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The Journal of Parasitology

Volume 5 DECEMBER, 1918 Number 2

NOTES AND EXPERIMENTS ON *SARCOCYSTIS TENELLA* RAILLIET

II. SEASONAL INFECTION

JOHN W. SCOTT

In a former paper (1915) the writer stated some evidence favoring Darling's suggestion that Sarcosporidia are merely aberrant varieties of the Neosporidia of certain invertebrates. At the time it was mentioned that experiments were in progress to test the question whether this hypothesis was correct or not. Results so far have shown that the control lambs were not entirely free from infection, so a report on these experiments will be deferred until a later paper. Certain definite data have, however, been obtained with reference to the time of year and other conditions under which infection does and does not occur. The present paper will discuss such data. By using more rigid methods of examination it has been found that apparently 100 per cent. of all adult range sheep are infected with sarcocysts. In the work reported here the lambs examined for sarcocysts were raised under various conditions. Except as otherwise stated the experimental lambs were kept in a dry lot from the time of birth; were supplied with city water which comes from deep springs, and were fed baled native hay kept over from the previous season. Those lambs over which there was no control are referred to as range lambs. By a reference to Table 1 it will be noted that in all groups where grazing was involved 100 per cent. of the lambs became infected. This was true whether the lambs were grazed on the range, in dry or wet pasture, or were fed grass, or flowers, taken from the wet pasture (Groups 1, 2, 4, 5, 6). It is also true that seven out of nine of the control lambs became infected (Group 3). Group 8 was treated as control lambs except that each of the eight lambs used was fed twice weekly a different kind of insect. Six out of eight of these lambs became infected. However, two of these lambs had a very heavy infection, in fact, heavier than any of the groups listed except Group 1, which included the range lambs. A full account and a discussion of the significance of these results will be given at another time. They are mentioned here to show the different conditions under which the lambs were raised from which some of the important data for this paper was derived, and to indicate that one probably should not expect entire uniformity with respect to the time or degree of infection.

TABLE 1.—TO SHOW INFECTION UNDER DIFFERENT CONDITIONS

Groups	Number in Group	Treatment	Number Infected	Per Cent. Infected
1	5	On range until killed	5	100
2	6	Wet pasture, July 15 to September 23	6	100
3	9	Control. Kept in dry lot; no green food	7	77.7
4	6	Dry lot; grazed twice weekly in lot around pond	6	100
5	2	Dry lot; grazed twice weekly in dry grassy pen near pond	2	100
6	2	Dry lot; fed grass and flowers from wet pasture twice weekly	2	100
7	1	Dry lot; watered twice weekly from pond of Group 4	1	100
8	8	Dry lot; each fed a different kind of insect twice weekly	6	75

Group 1 consisted of early spring lambs; other groups were all May lambs. In all lots ewes ran with their lambs.

Of the lambs grouped in Table 1, three of the range lambs were killed July 13, and the other two six weeks later on August 24. Of the experimental lambs, six were killed October 27; six on November 3; six on November 10; five on November 17; six on the following January 13, and five about two months later, on March 16. In each of these lambs the tip of the heart muscle was saved, fixed in absolute alcohol, embedded, cut in serial sections 16μ in thickness, and stained with Delafield's hematoxylin and eosin. This treatment brings out the sarcocyst in sharp contrast to the muscle tissue in all except the earlier, or Bertram's stages, to find which one needs to use a high power and mechanical stage to avoid overlooking them among the muscle nuclei. The work has involved the preparation of several hundred slides. Altho infection is generalized, the tip of the heart was used for examination, since it is easily localized and conditions are uniform.

The sarcocysts vary considerably in shape, dependent chiefly upon location in the body, and partly upon arrangement of the muscle fibers and connective tissue. For example, in the diaphragm the sarcocysts may be much elongated, and may approximate a spindle shaped form. The typical sarcocyst in the heart, however, is circular in cross section and in longitudinal section is approximately the form of an ellipse. In order to readily obtain a measure of volume it was decided to square the mean diameter and multiply this result by the length of the sarcocyst. This product gives the volume of a rectangular solid just large enough to enclose the sarcocyst measured, and generally is very nearly proportional to the volume. Some sarcocysts were too irregular in shape to obtain an accurate measure of volume, and these were excluded from the calculations. In early stages the length is usually several times the diameter, but later the diameter increases more rapidly than the length. All measurements were made by means of an eyepiece micrometer, and calculated in micra.

In working over the material to determine whether infection had occurred under the various conditions enumerated above, it was observed that the older the lamb the larger was the sarcocyst; this was to be expected. It was also noticed that in those lambs killed in January and March small sarcocysts appeared to be very scarce or entirely wanting. This seemed to indicate that infection did not occur during the winter and suggested a number of problems. Is infection continuous or discontinuous? Does infection occur only in young lambs? That is, does a range lamb become infected for all time while it is a lamb? Or, is infection seasonal, and does it recur year after year? In an attempt to answer these questions it was decided to take careful measurements of all sarcocysts found in these experimental lambs, and in other lambs and old ewes killed at various seasons of the year.

TABLE 2.—TO SHOW THAT THE AVERAGE SIZE OF THE PARASITES INCREASES WITH THE AGE OF THE LAMB

Group	Date Killed	Lambs Examined	Sarcocysts Measured	Mean Diameter	Mean Length	Mean Volume × Mean D^2 × Mean L.
1	July 13	3	29	29.7	101.7	89,708
2	Aug. 24	2	20	29.6	119.5	104,701
3	Oct. 27–Nov. 17	19	109	30.4	106.3	98,238
4	Jan. 13	6	51	34.5	120.4	143,306
5	Mar. 16	5	39	41.9	110.6	194,170
6	Oct. 27–Nov. 17	3	17	28.3	101.2	81,050
7	Jan. 13	3	25	34.0	125.5	145,078
8	Mar. 16	1	9	48.8	100.3	238,858

Measurements are given in micra. Group numbers do not correspond to groups of Table 1.

A reference to Table 2 shows that the average size of the parasite increases with the age of the lamb. A brief explanation will make this clear. The first column gives group numbers; the second, the dates when the groups were killed; the third column gives the number of lambs examined, and the fourth the number of sarcocysts measured in each group; in the fifth and sixth columns are found the mean diameter and mean length of each group of sarcocysts; in the seventh column is found the mean volume of each group of sarcocysts given in cubic micra; this volume was obtained by squaring the mean diameter and multiplying by the mean length. Groups 1 and 2 were early spring range lambs, the exact age of which is not known; they were, however, as large in August as the experimental lambs were in October, and were probably born in March or early April. Comparing these two groups, killed just six weeks apart, we find the mean volume of the sarcoysts has increased from 89,708 cubic micra on July 13 to 104,701 cubic micra on August 24. Groups 3, 4 and 5 were late April and May lambs; these were all kept in a dry lot until July 15, when there was begun the experimental work noted above. Groups 4 and 5 were killed at about nine week intervals after Group 3. Here too we find a gradual increase in the mean volume of the parasites. Groups 6, 7 and 8 represent infected control lambs, the data from which are included under Groups 3, 4 and 5, respectively; the data for the control lambs are given separately to show that the same general fact holds in lambs receiving identical treatment and killed at different times.

The fact is also brought out in Table 2 that the mean length, or mean diameter, of a group of sarcocysts is not necessarily proportional to the age of the lamb. In other words, the mean diameter, or mean length, is a variable dependent upon the nature of the tissue in which the sarcocyst is located. The amount of growth increases with the age of the lamb, but the direction of growth is a function of the tissue and depends upon other factors than age. There is also evidence to show that the rate of growth is faster in some lambs than in others; and that sarcocysts are retarded somewhat in growth if they chance to find lodgment in a location unfavorable for nutrition. Since the direction of growth may take place in three directions and since the sarcocyst reproduces by spore formation, it is to be expected, other factors being equal, that the volume of a sarcocyst will increase in a geometrical ratio with arithmetical increments of time. Such data as are available strongly indicate that this conclusion is correct.

That the mean volume of the sarcocysts would increase with the age of the lamb was to be expected. One would anticipate this result whether infection is a continuous or a discontinuous process. Now if infection is continuous, in lambs killed at successive intervals, one may expect the ratio of the volume of the smallest sarcocyst to the volume of the largest sarcocyst to increase gradually with the age of the lamb. But if infection is discontinuous, one may expect this ratio to increase until after the infective period is over and then gradually decrease, until after the time arrives for reinfection.

TABLE 3.—TO SHOW THAT INFECTION IS DISCONTINUOUS

Group	Date	Number Lambs Used	Sarcocysts Measured	Mean Volume of Parasites	Ratio of Volume Smallest : Largest	Ratio of Volume Smallest : Mean
1	July 13	3	29	89,708	1 : 70.18	1 : 11.95
2	Aug. 24	2	20	104,701	1 : 75.87	1 : 18.21
3	Oct. 27-Nov. 17	19	109	98,238	1 :109.76	1 : 18.60
4	Jan. 13	6	51	143,306	1 : 9.71	1 : 2.43
5	Mar. 16	5	39	194,170	1 : 8.11	1 : 3.77
6	Oct. 27-Nov. 17	3	17	81,050	1 : 21.08	1 : 9.42
7	Jan. 13	3	25	145,078	1 : 3.09	1 : 1.91
8	Mar. 16	1	9	238,858	1 : 6.00	1 : 3.42

Same groups compared as in Table 2. Note that while the mean volume of the sarcocyst increases with the age of the lamb, after a considerable period the ratio of the smallest to the largest parasite decreases instead of continuing to increase.

A study of Table 3 shows that this in general is what one finds. The ratio of the smallest to the largest of twenty-nine sarcocysts killed on July 13 was 1 to 70.18. By August 24, of twenty sarcocysts measured, the ratio had increased to 1 to 75.87, thus indicating continuous infection. These ratios probably do not give the best sort of a comparison, for if one leaves out of consideration the largest parasite for each date, the ratios become, on July 13, 1 to 35.18 and on August 24, 1 to 57.79. It should be mentioned that the smallest parasites found for these dates had about the same volume. Or, if one takes the ratio of the smallest parasite to the mean volume, it becomes for July 13, 1 to 11.95, and for August 24, 1 to 18.21. In any case both the ratios and the mean volume increase, while the size of the smallest parasite found does not increase. Consequently, infection has been continuous throughout this period.

Now in the experimental lambs, killed October 27 to November 17, the smallest sarcocysts found were about the same size as the smallest found during the summer, but the ratio of the smallest to the largest at this date is 1 to 109.76, and the ratio of the smallest to the mean is 1 to 18.60. Comparing these smallest stages found with the stages figured by Erdmann (1910), it is probable that they represent an age 5 to 7 weeks after infection, and from 1 to 3 weeks after invasion of the musculature. It is probable therefore that infection occurred in these lambs up to about October 1. It is also interesting to make a comparison with the results obtained by killing lambs on January 13 and March 16. The smallest parasite found on these dates had volumes between 50,000 and 60,000 cubic micra, while the smallest parasites found during the summer and fall were only one-ninth or one-tenth as large. On January 13 the ratio of the smallest to the largest parasite was 1 to 9.71, and its ratio to the mean was 1 to 2.43. So one finds that a large decrease in the ratio has occurred in spite of a large increase in volume, and this decrease is due to the fact that small sarcocysts are no longer found. The same holds true on March 16. Here the ratio of the smallest sarcocyst to the largest is 1 to 8.11; that is, the ratio is still decreasing. The ratio of the smallest to the mean volume is 1 to 3.77. This is somewhat larger than the corresponding ratio for January 13, and is accounted for by the fact that the smallest parasite found in these lambs on March 16 had about the same volume as the smallest one found on January 13. A larger series of measurements for these dates would no doubt render more uniform results. I have proof to show that this would be true. In an old ewe killed December 28 the smallest sarcocyst had a volume of 38,304 cubic micra. Considering the rate of growth, one would expect the smallest parasite found on January 13 to have a volume between 40,000 and 50,000 cubic micra, instead of 58,000 as was the case in the few experimental lambs killed on this date. Groups 6, 7 and 8 represent the results from the control lambs, given separately. Here too the results, to a certain extent, lack uniformity, due to the small number of parasites involved. However, in all cases *it is clear that infection has been discontinuous,* and probably there was *no infection after about the first of October.*

Another question, with reference to how early in the spring does infection begin, has not been so accurately determined. If all sarcocysts had the same rate of growth, one could measure the largest sarcocysts found in lambs at different dates during the summer, and could determine fairly accurately the approximate time when infection begins in the spring. The rate of growth, however, of a particular sarcocyst appears to depend to a considerable extent upon the amount of nutrient fluid, and pressure of the tissues, surrounding it. It is noticeable that the larger sarcocysts of a lamb of given age killed during the winter are in the looser, more vascular portions of the heart muscle. Likewise the smallest sarcocysts of such a lamb are found in the more compact and denser regions of the heart tissue. Consequently to draw conclusions from the study of a few sarcocysts will not be satisfactory, and up to this time there has been no opportunity to study a large body of controlled material taken in the spring or summer. Still, some of the results are interesting.

An old ewe, No. 226, was killed June 13. A very careful examination showed that the smallest sarcocyst found had a volume of 102,513 cubic micra. Evidently this parasite belonged to infection during a preceeding season, for one would expect to find much smaller sarcocysts if infection had begun the current spring. Again, the smallest sarcocysts found in March (Group 5, Table 3) had volumes between 53,000 and 60,000 cubic micra, and one would expect these to have a volume in June of over 100,000 if they increased in size at an average rate (see Table 3 for average rates of growth). So if one allows six weeks for the infection to appear in the muscles, reinfection had not begun (Ewe 226) on May 1. However, one must not overestimate this evidence for only twelve sarcocysts were found in the material preserved from this ewe; she had had small chance of infection recently and she had not been outside of a dry feed lot for more than twenty months. Perhaps if this ewe had been running on the range some small sarcocysts would have been found.

Two lambs, 986 and 996, one born March 26, and the other April 3, were killed June 22. No sarcocysts were found in either of these lambs. For a considerable portion of the latter part of April and during May they had access to pasture conditions. Beginning the first week in June they were kept in a dry lot until killed. If infection occurs without the necessity of any other host, one would expect to find sarcocysts in these lambs, since they were both old enough to show the infection and had been exposed to pasture conditions. Incidentally, it may be mentioned that, owing to a late season, practically no insects were present up to the date when they were killed. Lamb 302, born early in June, died July 20. On June 13 this lamb had been taken from the ewe to be raised by hand, and had no contact with sheep after this date. It did not begin to eat grass to any extent until about the end of June, and if it became infected, the sarcocysts had not yet appeared in the muscles. Lamb 286, born about the middle of May, was kept in a dry lot until it died, July 28. No sarcocysts were found. It had been fed certain insects on July 17, 20, 24 and 27. Conditions for infection were not favorable, and this negative result is of no value. Lambs 284 and 294 born in May were killed August 9. They were kept in a dry lot until July 16, after which they were turned out to graze in a pasture where 100 per cent. infection had always been obtained in all lambs. These lambs, had, therefore, probably become infected, but the sarcocysts had not yet appeared in the heart muscle twenty-four days after their first exposure to favorable conditions for infection. Lambs 283 and 291, born in May, were killed September 22. These lambs were treated like 284 and 294, but were allowed to run in the pasture forty-four days longer, a total of sixty-eight days in pasture. In the material sectioned, one sarcocyst with a diameter of 15.4 and a length of 44μ was found in the heart muscle of lamb 282. So far as studied, no sarcocysts were found in lamb 291. The size of the sarcocyst indicates that this infection could not have occurred much, if any, later than Agust 1, assuming it takes forty days for the parasite to become established in the muscle tissue. The evidence presented above with reference to how early in the spring infection begins, is of no great value. However, it appears that infection does not begin very early in the spring, and probably at least five or six weeks must intervene between actual infection and the appearance of the sarcocysts in the muscle tissue. Altogether, *it is very clear that there is a distinctly marked seasonal infection,* and this fact is in agreement with the idea suggested in a former paper that *Sarcocystis tenella* may be primarily dependent upon some invertebrate, probably some insect host. But the solution of the question whether Sarcosporidia are aberrant forms of Neosporidia as maintained by Darling (1915) and supported by Scott (1915), or are identical with Cnidosporidia, as suggested by the observations of Piana (1896) and Galli-Valeria (1916), must depend upon other data. It is believed that certain experiments now in progress will throw light on this problem.

Since infection is hard to control, and since practically all sheep of this region are infected, the theory has been suggested that infection was possibly transmitted *in utero*. There is strong evidence against this idea. A lamb, No. 301, died shortly after birth. The mother was killed soon afterward and proved to be an old heavily infected ewe. No sarcocysts were found in the lamb. In our experiments other older lambs, from infected mothers, have also been found free from infection. In this connection considerable work has been done. Bertram (1892) examined the embryos of sheep, swine and cattle, but found no sarcocysts in any of them.

Bergmann (1913) found sarcocysts in 8 per cent. of lambs 6 to 10 weeks old, but he found that 20 per cent. of lambs just a little older (3 months) were infected. M'Gowan (1914), who believes in congenital infection, examined in 1913 "a large number of embryos from heavily infected mothers," but nevertheless found no infection; he also examined a number of lambs at various ages and the earliest age at which he found infection was 3 months. In the following year he made a more critical examination, by means of serial sections, of lambs 2, 16, 18, 22, 27 and 29 days old, but found no sarcocysts. All the evidence is against the theory that infection occurs *in utero*. The gestation period of the sheep is nearly five months, and considering the cotyledonous type of placenta at least ten or twelve weeks of the period should be favorable for infection. If six weeks is added to the period favorable for infection, since no lambs under 6 weeks have been found infected, sixteen or eighteen weeks appears as probably the shortest time from infection until the appearance of the parasites in the muscles. But in the case of *S. muris,* according to Smith and Nègre, the time from infection to appearance of the parasites in the muscles may be as short as forty-five days, and there is no apparent reason why the time for *S. tenella* should be longer. Now Bergmann is the only investigator who has found sarcocysts in the muscles of lambs at an age approximating this short period, namely in lambs 6 to 10 weeks old. It then appears that infection after birth agrees with all facts yet brought forward, and all the evidence is opposed to the idea of infection before birth.

One may now consider the question, does infection occur only in young lambs, or does it recur in successive seasons? The proof is apparently conclusive that the latter alternative is correct. In the old ewes one finds small sarcocysts, and in addition large ones that are immensely larger than any of those found in lambs during the first year. This proves that infection extends over more than one year; that is, infection may and probably does recur in successive seasons as long as the sheep lives. An inspection of Table 4 will help to make this matter clear.

In the case of Ewe 226, killed about the middle of June, the volumes of the nine parasites given apparently fall in three or four groups, and so probably represent infection in three or four different seasons or years. Parasites 1 to 4 no doubt belong to infection one year back; parasites 6 to 8 are probably in their second or possibly third year; and parasite 9 is separated widely from number 8, and is seemingly of older growth, perhaps about 3 or 4 years old. Since the largest sarcocysts found in lambs killed in March (Group 6, Table 3) were less than 600,000 cubic micra, sarcocyst 5 may be only about one year old, but it probably belongs to the second season preceding. However, one cannot determine with certainty the age by the size of the parasite, after the first year, on account of the different rates of growth of different sarcocysts. At the end of the second year the largest parasites of that year are overlapping in size the smallest due to infection the first year. By accumulating a vast amount of data one could possibly determine the probable age of each sarcocyst. Ewe 226 was more than 3 years old, but her exact age is not known.

TABLE 4.—TO ILLUSTRATE THE VOLUMES OF SARCOCYSTS IN OLD EWES AND TO SHOW THAT INFECTION EXTENDS THRU MORE HAN ONE SEASON

Sarcocyst Number	Ewe No. 226 Killed June 13	Ewe No. 4 Killed October 24	Ewe No. 717; Killed December 28		
	Volume	Volume	Volume	Sarcocyst Number	Volume
1	102,513	4,702	27,684	51	298,144
2	182,023	5,712	30,178	52	299,625
3	185,526	6,701	38,304	53	309,217
4	185,856	6,751	42,592	54	327,703
5	644,335	9,820	45,999	55	332.750
6	1,018,484	18,817	57,584	56	332,750
7	1,354,896	56,453	61,327	57	340,139
8	1,741,610	90,626	64,420	58	383,029
9	2,861,932	93,828	71,874	59	404,624
10	....	96,341	89,975	60	412,280
11	....	126,737	92,248	61	417.404
12	....	133,816	119,700	62	439.357
13	....	134,853	119.700	63	446,880
14	....	136,294	119,700	64	450,410
15	....	137,773	122,998	65	453,508
16	....	146,232	122,998	66	484,183
17	....	147,581	124,104	67	519,090
18	....	156,119	125,390	68	545,177
19	....	157,767	125,390	69	578,985
20	....	169,458	130,842	70	622,908
21	....	172,497	131,098	71	665,000
22	....	180,495	131,769	72	704,365
23	....	234,890	137,998	73	709,582
24	....	249,356	143,748	74	776,354
25	....	275,272	143,748	75	795,659
26	....	283,947	144,472	76	851,840
27	....	297,369	144,812	77	862,488
28	....	311,364	146,143	78	863,755
29	....	325,075	153,863	79	875,844
30	....	338,875	172,327	80	1,042,720
31	....	372,956	172,491	81	1,120,234
32	....	377,013	172,497	82	1,225,017
33	....	429,143	179,685	83	1,245,816
34	....	453,955	179,902	84	1,333,312
35	....	456,744	180,241	85	1,596,000
36	....	543,250	183,144	86	1,697,376
37	....	561,009	184,497	87	1,770,602
38	....	732,674	189,747	88	1,788,864
39	....	754,252	190,812	89	1,862,400
40	....	765,215	193,197	90	1,931,160
41	....	766,656	196,196	91	2,793,100
42	....	766,656	204,441	92	2,863,436
43	....	1,072,599	206,841	93	3,194,400
44	....	1,384,825	212,960	94	3,194,400
45	....	1,397,439	225,142	95	4,599,936
46	....	1,893,959	245,326	96	5,398,536
47	....	1,992,623	250,360	97	6,673,340
48	....	....	268,329	98	6,965,977
49	....	....	281,107	99	9,171,411
50	....	....	281,107	100	16,959,640

From range Ewe 4, killed October 24, there was obtained a larger series of sarcocysts. A comparison of the volumes of the forty-seven sarcocysts measured with the volumes of the sarcocysts of the experimental lambs shows that the parasites of this ewe probably owe their origin to infection during three different seasons. Sarcocysts 1 to 22, and possibly 23 and 24, belong to the summer just past; sarcocysts 25 to 42 are probably in their second year, while 44 to 47, and probably 43, are in their third year. In any event the measurements show that infection has taken place in more than one season. No information was obtainable in regard to the age of this ewe. In reference to this group of sarcocysts another fact is of importance. The smallest sarcocysts found (Table 4) are about the same size as the smallest sarcocysts found in the experimental lambs killed October 27 to November 17. That is, infection occurs about as late in the season in old ewes as it does in lambs.

A still larger series of parasites was measured from Ewe 717, which was killed on December 28 at the age of 3 years and 9 months. This ewe had, therefore, been exposed to four seasons of infection. By a comparison with the results obtained with the lambs killed at different dates, the average size of the parasites of the fourth, that is, the immediately preceding summer, should be about 132,000 cubic micra. By a comparison with the largest sarcocysts found in lambs killed January 13, parasites 1 to 45 (Table 4) all belong to this fourth season. By a similar comparison, parasites 46 to 79, inclusive, with volumes varying from 245,326 to 875,834 cubic micra, should probably be assigned to the third, or second, preceding season. Sarcocysts 80 to 94, with volumes varying from 1,042,720 to 3,194,400 cubic micra, would seem to belong to the second, or third, preceding summer, and parasites 95 to 100, inclusive, probably belong to the first, or fourth, preceding season of infection. Here too it is evident that infection extended through more than one season. A study of Table 4 brings out another point of interest. If ewe 717 was infected during four different seasons as it had a chance to be, then there could be four groups of sarcocysts with approximately the same number in each group. But if these observations are based upon adequate data and the reasoning has been correct, it appears that the older the group, the smaller the number of sarcocysts in that group. This has been found to be true in other cases besides the three ewes given, and may be accounted for in one of three ways. Either the sarcocysts gradually disappear by disintegration, as they grow older; break up into smaller sarcocysts; or some of the older sarcocysts never reach more than a moderate volume. I have no proof for or against the first alternative, there is some evidence against the second, and certain observations indicate the third view is correct. After examining many hundreds of sarcocysts I cannot say that I have found any evidence that sarcocysts break up by disintegration. If older sarcocysts break up, thus setting free spores which wander out and become seats of new infection, this procedure in the light of facts brought out in this paper, must be an annual periodic occurrence. Aside from the lack of plausability, the proportion of small to larger sarcocysts is not compatible with such a theory. While occasionally a sarcocyst shows a stricture on one side or at one end, I have never found such a stricture approach complete separation, nor does one find sarcocysts in pairs or in fours as one would expect if such fragmentation or division of the sarcocyst did occur. Fantham and Porter, referring to their figure 55 which represents a dozen or more young pansporoblasts, state that "at this stage pansporoblasts (sometimes called sporonts) may wander out and start new infection." Even if this is true, all sarcocysts pass far beyond this stage before the second summer approaches, and another explanation will need to be given for seasonal infection. Finally, in support of the third view it has been shown that in lambs of known age the size of the sarcocyst bears a close relation to the nature of the tissue surrounding it. This is true for the first season of infection, and it would seem reasonable to believe it holds true for later stages of growth. In this way one can account for the apparent gradual decrease in the number of parasites in the older groups. Another fact supporting this view is that in some old ewes the number of sarcocysts seemingly derived from the last preceding season of infection appears rather large as compared with infection in lambs that have passed thru only one summer. In any event, it is evident that *ewes become infected year after year in successive seasons.*

TABLE 5.—TO SHOW DIMENSIONS OF SOME OF THE SMALLEST SARCOCYSTS MEASURED

Number of Lamb or Ewe	Date Killed	Diameter, Micra	Length, Micra
Range lamb 3	July 13	11.0	57.2
Range lamb 5	Aug. 24	13.2	33.0
Exp. lamb 283	Sept. 22	15.4	44.0
Range ewe 4	Oct. 24	6.6	107.9
Range ewe 4	Oct. 24	8.8	61.6
Range ewe 4	Oct. 24	9.3	78.0
Exp. lamb 280	Nov. 3	9.4	68.0
Exp. lamb 263	Nov. 10	11.0	44.0

Perhaps a mention should be made of the size of some of the smallest sarcocysts found. The dimensions given in Table 5 above will serve as a basis of comparison with the findings of other investigators. The smallest sarcocyst of *S. tenella* observed by Bertram was 6μ wide and 47μ long. This is smaller than any of those given in the table, but all of those given were apparently in the so-called Bertram stage. Crawley claims to have found a cyst consisting of a single dividing sporoblast, and another which contained eight sporoblasts, both located intracellularly. This apparently proves that the elements in a sarcocyst undergo schizogonous multiplication. Fantham and Porter have seen all stages in this division which occurs by longitudinal fission, and they describe the growth and extension of a Sarcosporidian in a vertebrate host as follows: "Each spore contains an amoebula which finds it way into a muscle. The amoebula grows and its nucleus divides, thus becoming an elongate, multinucleate mass. Around each nucleus the protoplasm segregates, and a number of young pansporoblasts are formed. At this stage pansporoblasts (sometimes called sporonts) may wander out and start new infections (Fig. 55). Later, partitions or septa are formed between the pansporoblasts. Several spores are ultimately found in each chamber, having been formed from the pansporoblast." Judging from this description, and their figure 55, the stage at which migration may occur is the so-called Bertram's stage of various writers. The sarcocysts listed in Table 5 were all approximately in this stage, tho two or three showed the protoplasm had not yet become definitely segregated around the individual nuclei. From the data available from all sources it is probable that these parasites in Table 5 represent stages from six to nine weeks after infection.

DISCUSSION AND SUMMARY

Reference has already been made to the report of M'Gowan (1914) who wrote this lengthy paper with the purpose of showing that the disease "scrapie" is associated with and probably is caused by Sarcosporidiosis. Tho a careful reading of his paper leaves one unconvinced, this thesis leads him to postulate a partial theory in reference to the life history of *S. tenella.* As stated above, a part of this theory involves infection *in utero,* and I have presented evidence to show that this is certainly not the usual method of infection. Aside from the data which demonstrate recurring seasonal infection, the youngest age at which *S. tenella* has been found in lamb muscle is amply sufficient to account for infection after birth. No one has found sarcocysts present in lambs under 3 months old, except Bergmann, who found them in a small percentage of lambs that were somewhere between 6 and 10 weeks old. One does not know just how soon after infection *S. tenella* may appear in the muscles, but it is known (Smith, Nègre, Erdmann, Crawley) that *S. muris* appears in the muscles 40 to 50 days after infection, and there is no reason why *S. tenella* should require a longer period. Many facts are opposed to the idea of infection *in utero,* and not a single fact has been adduced incompatible with the theory that infection occurs after birth. In this connection it may be well to examine the experiment that M'Gowan gives as a crucial test.

In April and May (1913) "four lambs, from four scrapie sheep, were obtained *as soon as they were born,* before the mothers had even licked them. They were removed at first to a large byre where no sheep had ever been before, and later to a field where a similar condition prevailed. No sheep were ever allowed near them. They were looked after by an attendant whose duties did not bring him in contact with other sheep. They were brought up on cow's milk until they were old enough to live entirely on grass. When they were about one month old, living keds from scrapie sheep were applied to two of them, and these two were kept apart from the others. No further step was taken until January, 1914, in order that if sarcocysts did develop there would be no doubt of their actual presence. Then pieces of muscle were examined from the gluteal region of all four, *and in all four fully-developed sarcosporidial cysts were found in as large numbers as in lambs from scrapie mothers and of the same age brought up under natural conditions.*" . . . "and from the experiment it would appear that no conclusion could be drawn from it other than that the parasite is passed on by congenital infection of the lamb from its mother."

This conclusion might be justified if the experiment did not admit of other explanations. If Darling's insect theory of infection is correct, one would expect just as heavy infection in these lambs as in lambs which ran in fields with their mothers. Again, if infective spores are set free in the feces, as is true of *S. muris,* the possibility of infection in this manner is not entirely excluded. For, some one *brought the living keds from scrapie sheep,* and evidently here was a chance for infection by contamination. While this experiment shows that sarcosporidial infection takes place independent of the sheep tick, it affords no evidence that cannot be explained as well, or better, by theories of infection after birth.

Fantham and Porter are quoted above with reference to an early stage in the development of a sarcocyst at which the pansporoblasts may wander out and start new infections. Granted this is a common method of multiplcation, it will not serve to account for seasonal reinfection, for the sarcocysts have passed beyond the pansporoblast stage before the second season arrives. It is hardly probable, that this is the usual method of reinfection, since one would not expect such an internal method of multiplication to cease on the approach of winter. M'Gowan's theory of multiplication and reinfection is even less probable. According to this author, the chromatin granules in a spore escape by bursting of the spore, and play a part in transmission of the disease (scrapie) both endogenously and exogenously. Our knowledge of the Sarcosporidia indicates that the spore is the unit of infection, and even if his theory were possible it would be hard to reconcile it with what is now known of the seasonal character of infection and reinfection. On the whole, considering in particular the work of Nègre, Erdmann and Crawley, it would appear that the source of infection is external and by the way of the alimentary canal. The experimental evidence brought out in this as well as in a preceding paper is in favor of the same conclusion. In fact, it is hard to see how seasonal infection could depend upon anything other than external conditions, either directly or indirectly, and since it is known that certain sarcosporidial spores enter the body by way of the alimentary canal, this is most likely the usual avenue of infection.

M'Gowan examined a large number of sheep and lambs, and incidentally produced some data that correlates with seasonal infection. Between January 21 and April 29, 1913, he found sarcocysts in 553 out of 818 sheep (67.6 per cent.), all of which were about one year of age or older. This shows that infection is the prevalent condition among sheep of that region. Between April 30 and June 11 he examined 121 February lambs and found sarcocysts in only four, or 3.3 per cent. Allowing forty to fifty days after infection for the sarcocysts to appear in the muscles, it is probable that this percentage would have been larger if there were no external conditions influencing the time of infection. At the same time the data agrees nicely with the idea of seasonal infection.

A discussion of some of the larger aspects of the relation of seasonal infection to the life history of *Sarcocystis tenella* will be deferred until a later paper. It seems entirely probable that infection occurs by way of the alimentary canal. It is clear that sheep at any age are susceptible to infection, and seasonal infection does not appear to be due to any condition within the sheep's body. Being so, do these conditions depend directly upon climatic factors, such as temperature? Or do they require an intermediate host present only at certain times of the year? The answer to these questions will depend much upon the answer to what is the life history of the parasite outside of the muscles of the host, a question which is yet unanswered. Successive seasonal infection is fatal to the theory of infection *in utero*. If there is a stage, or phase of *S. tenella* in the intestine which results in the freeing of spores, as Nègre (1907) has shown to be true of *S. muris*, it would seem that temperature may be a direct prominent factor in controlling seasonal infection. And yet since the encysted spores remained alive for thirty days in dried feces and resisted a considerable degree of heat, it does not seem possible on this theory to explain the entire absence of winter infection in *S. tenella* unless the spores are extremely sensitive to cold, which is improbable. There is no other known climatic factor in the region of the Laramie Plains that could bear a direct causal relation to seasonal infection. Equally improbable on the same grounds is the theory that a carnivorous intermediate host is necessary. Other facts opposed to the latter view were presented in a former paper.

It would seem then that seasonal infection bears only an indirect relation to climate. If so, the factors that determine infection in turn must depend upon climate. To fulfil these conditions an intermediate or more likely, a definitive host is required. Crawley (1916) believes the Sarcosporidia should be classed under Telosporidia rather than as Neosporidia, basing his conclusion on what is now known of the life history of *S. muris* and certain young stages that he found of *S. tenella*. More needs to be known of the life history of these forms before this question can be settled definitely. Considering the widespread occurrence of Sarcosporidia in herbivorous animals, such as the sheep, Crawley also believes a second host is obligatory. This is probably correct, but his hypothesis with reference to a carnivorous animal must be rejected. Under the conditions of our experiment it is not possible to account for such infection as I have obtained on the theory of a carnivorous intermediate host, whether it be dog, cat, rat, mouse or ground-squirrel. If then a second host is necessary for *S. tenella,* the best remaining hypothesis is to look for this host among the invertebrates and a former paper has given reasons for believing this host would be found among the insects. The seasonal dependence of insects is also in accord with the facts presented in this paper.

There is a second hypothesis that must be taken into consideration. Nègre has shown that the feces of a mouse infected with *S. muris,* become and remain infective for a long time, from the fifteenth to the sixtieth day. Crawley has verified this work, and while the bodies that cause the infection have not been actually observed, they are known to exist in the feces. If there is a similar stage in the life-history of *S. tenella* the fragile character of the spores, which has been noted by Fantham and Porter, may be sufficient to account for seasonal infection. The dry, cool climate of this region, frequently becoming quite cold in the winter may soon kill the spores during a portion of the year. However, there are many mild periods in winter which should not be very destructive, and the lambs of the experiments noted in this paper ran, fed and watered with their ewes, so they had abundant opportunity to become infected by contamination. Yet there was not a single case of winter infection. Either the spores are set free at only certain seasons, or there is some doubt in regard to this second hypothesis. Experiments are now in progress which will probably show which of the two hypotheses is more nearly correct.

SUMMARY

The chief points of this paper may be summarized as follows:

1. There is a well-defined seasonal infection of *Sarcocystis tenella* in the region of the Laramie Plains. It is not known whether this is true or not of other regions. Young stages of this parasite have been found in the muscles of both sheep and lambs thruout summer and early autumn, but not during the winter and spring.

2. Reinfection occurs in successive seasons, and old sheep are apparently as susceptible to infection as are young lambs. The theory of infection *in utero* is untenable. Seasonal, self-reinfection is improbable, tho not entirely excluded, and the evidence indicates the origin *de novo* of successive infections.

3. If a second host is required, which seems probable, it is very likely that this host is an insect, and that the definitive (sexual) stage of the parasite will be found here.

4. If a second host is not necessary, the sexual stage probably takes place in the intestine of the sheep, and in some unknown way the life cycle falls under the influence of seasonal control.

5. In old ewes the larger sarcocysts are not nearly so abundant as the smaller ones. That some of the older sarcocysts do not grow to a large size is probably the most satisfactory explanation of this fact.

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